

TWO SAINFOIN (*ONOBRYCHIS VICIIFOLIA* SCOP.) CULTIVARS DIFFER IN THEIR RESPONSES TO NEUTRAL AND SALINE-ALKALI STRESS DURING SEED GERMINATION AND EARLY SEEDLING GROWTH

LI, S. J.¹ – ZHU, Y. H.¹ – WHITE, J. F.² – WEI, M.¹ – WU, G. Q.^{1*}

¹*School of Life Science and Engineering, Lanzhou University of Technology,
Lanzhou 730050, China
(phone: +86-931-297-6650; fax: +86-931-297-3367)*

²*Department of Plant Biology, Rutgers University, New Brunswick, NJ 08901, USA*

**Corresponding author
e-mail: gqwu@lut.edu.cn; phone: +86-931-297-6060*

(Received 27th Jun 2021; accepted 20th Sep 2021)

Abstract. Salinity is one of the major environmental factors limiting plant growth and development. Two cultivars (the native “Gansu,” GS; and the imported “Italian,” IT) of sainfoin (*Onobrychis viciifolia* Scop.) were exposed to two neutral salts (NaCl and Na₂SO₄) and two alkaline salts (Na₂CO₃ and NaHCO₃) at concentrations ranging from 0-300 mM. After seven days seed germination rates and radicle and hypocotyl lengths were compared. Exposure only to 50 mM neutral salts did not affect significantly relative germination rates compared to control (0 mM). In contrast, higher concentrations of neutral salts and alkaline salts significantly inhibited germination rates and reduced radicle and hypocotyl lengths. Although the two cultivars were visually similar in appearance and had similar patterns in response to saline and alkaline stresses, GS had relatively higher threshold germination concentration compared to IT, and the radicles and hypocotyls of seedlings grown under NaCl and Na₂SO₄ were longer than for IT, indicating that GS is more salt-tolerant. Under neutral salt stress, the recovery germination percentage increased with higher salt concentration. However, under alkaline salt stress, the recovery germination percentage sharply decreased with increasing salt concentration. These results suggest that the damage caused by alkaline salts is more severe than those caused by neutral salts. The present study provides novel insights into the responses of seed germination to different types of salinity in an important forage species.

Keywords: forage, germination rate, hypocotyl, radical, physiological response

Introduction

Abiotic stress seriously affects plant growth and development, resulting in crop failure and reducing average crop yields worldwide (Muscolo et al., 2014; Khalil et al., 2016; Partheeban et al., 2017; Niu et al., 2018). Salinity is one of the most important abiotic factors and impacts almost every aspect of plant biochemistry and physiology, from seed germination and seedling growth to the final production and yield from mature crops (Lu et al., 2010; Gebremedhn and Berhanu, 2013; Radic et al., 2019). Exposure of seeds to saline conditions may compromise subsequent seedling establishment (Albuquerque and Carvalho, 2003; Ahmadvand et al., 2012). Indeed, salinization has already affected over 800 million hectares of land, approximately 6% of the total land area worldwide (Munns and Tester, 2008; Wu et al., 2015a).

Assessing salt tolerance of seed germination is extremely important because successful establishment depends entirely on seed germination, and seeds with more

rapid germination under saline conditions are expected to establish faster and accumulate more biomass (Gorai and Neffati, 2007; Petrovic et al., 2016). One of the most common experiments to study seed germination sensitivity to salinity involves the application of NaCl, Na₂SO₄, Na₂CO₃, and NaHCO₃ to seed and seedling media (Zahra et al., 2011; Idris and Ali, 2015; Hu et al., 2018; Li and Zhao, 2018).

Sainfoin (*Onobrychis viciifolia* Scop.) is one of the most bloat-safe forage legume crops with high tannin levels, and it is renowned for its medicinal value to grazing animals (Mohajer et al., 2014). Recently, various studies have characterized the environmental preferences, agronomy, plant-microbial interactions, and crop protection of sainfoin (Okcu and Topaloglu, 2019). Sainfoin is an important forage species in agriculture preferred by farmers over other forage legumes. It is widely distributed throughout the arid and semi-arid areas of northern China, Europe, and Russia (Baldinger et al., 2014; Wu et al., 2017; Cirujeda et al., 2019). Since meat products have become more popular as people's living standards have improved, increasing forage crops will be crucial in increasing livestock production. However, in some areas, development of forage crops is negatively affected by abiotic stress. As a result, forage plants may become the focus of breeding programs, particularly for developing salt-tolerant varieties. For sainfoin cultivation, the most important problem is poor germination (Okcu and Topaloglu, 2019), which always results in undesirable establishment and, occasionally, in crop failure (Abbasdokht et al., 2014; Radic et al., 2019). Our previous studies showed that low salt (5-50 mM NaCl) did not affect plant growth of sainfoin, whereas high salt (100 and 200 mM) significantly limited its growth (Wu et al., 2017). Recently, it was reported that the damages caused by alkaline (Na₂CO₃) stress on the growth of sainfoin plants were more serious than those caused by saline-alkaline (NaCl: Na₂CO₃) stress (Wu et al., 2021). However, the studies on effects of neutral and alkaline salts on seed germination and early seedling growth of sainfoin have been rarely performed.

The objective of this work was to investigate and compare the effects of two common salt stress factors, neutral salinity (NaCl and Na₂SO₄) and alkaline salinity (Na₂CO₃ and NaHCO₃), on seed germination and seedling growth of two sainfoin cultivars. The results of the present study would not only provide novel insights into the responses of seed germination to different types of salinity in forage species, but also provide a practical basis for improvement and utilization of saline soil.

Materials and methods

Plant materials

This study was carried out in the Lab of Plant Physiology and Ecology at Lanzhou University of Technology, Lanzhou, Gansu Province, China, from March through December, 2019. The seeds of sainfoin (*O. viciifolia* Scop.) cultivars "Gansu" (GS, native breed) and "Italian" (IT, imported breed) were purchased from Lanzhou Nongfeng Seed and Seedling Technology Co., Ltd. and Gansu Mammoth Agriculture Co., Ltd., respectively. The seeds of these two cultivars are very similar and cannot be distinguished based on appearance. The masses of 1000 seeds of GS and IT were 23.03 and 21.07 g, respectively. Prior to the experiment, excessively large or small seeds were discarded, and seeds of uniform size were collected for the germination experiments. Seeds were kept in a cold chamber at 4 °C until the start of the experiments.

Germination test

A total of four salinity types (NaCl, Na₂SO₄, Na₂CO₃, and NaHCO₃) were applied. Seeds of GS and IT were exposed to two neutral salinities (NaCl and Na₂SO₄) and two alkaline salinities (Na₂CO₃ and NaHCO₃) at 0, 50, 100, 200, 250, and 300 mM during a seven-day period and their germination assessed.

Seeds were first surface sterilized in a 3% sodium hypochlorite solution for 8 min, then rinsed with the sterile distilled water five times, soaked in the distilled water for 12 h, and briefly dried on filter paper. Fifty seeds were randomly placed on 9-cm diameter Petri dishes with three layers of filter paper wetted with 12 mL of the treatment solutions. The filter paper and solutions were changed each day. Petri dishes were randomly placed in the dark in a precision incubator that maintained the temperature at 18 °C. Three independent replicates were conducted for each treatment.

After 7 d of salt exposure, ungerminated seeds were transferred to Petri dishes with only distilled water and incubated at 18 °C for an additional 6 d.

Data collection

Seeds were considered to have germinated when their radicle length was at least 1 mm. The number of seeds germinated on each Petri dish was recorded daily during the seven-day salinity exposure. The hypocotyl and radicle lengths were measured using a ruler on the seventh day. The germination rate (GR) was calculated according to Hu et al. (2018) as

$$GR(\%) = \frac{a}{b} \times 100 \quad (\text{Eq.1})$$

where *a* is the number of germinated seeds and *b* is the number of total seeds per treatment. The recovery germination percentage (RGP) was calculated as

$$RGP(\%) = \frac{c}{d} \times 100 \quad (\text{Eq.2})$$

where *c* is the number of newly germinated seeds in recovery test, and *d* is the number of seeds transferred to the distilled water treatment (Zhang et al., 2014).

Statistical analysis

Data analysis was performed using SPSS 22.0 (Chicago, USA). Two-way and three-way ANOVAs using salinity type, salinity concentration, and cultivar, as the factors were performed to analyze the differences among the four salinity types, the seven salinity concentrations, the two cultivars, and their interactions. Duncan's Multiple Range tests were used to determine significant differences between means at the *P* < 0.05 level.

Results

Effects of neutral and alkaline salts on seed germination rate

We compared the effects of different concentrations of neutral salinities (NaCl and Na₂SO₄) and alkaline salinities (NaHCO₃ and Na₂CO₃) on the time courses of seed germination (Figs. 1 and 2).

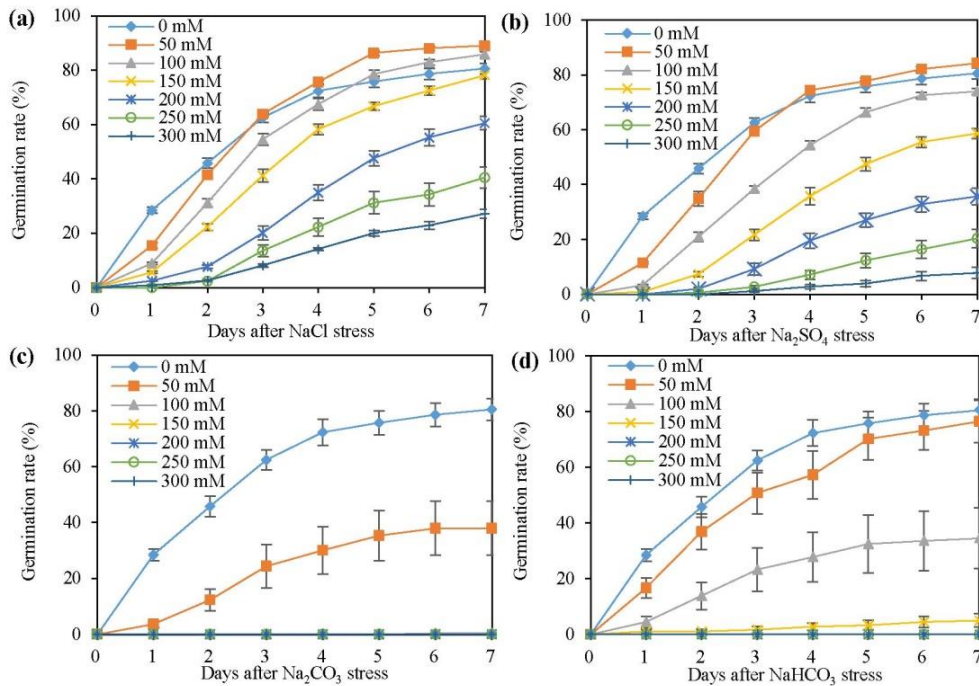


Figure 1. Time course of seed germination of the sainfoin cultivar “Gansu” (GS) subjected to NaCl (a), Na₂SO₄ (b), Na₂CO₃ (c), and NaHCO₃ (d) in various concentrations (0, 50, 100, 150, 200, 250, and 300 mM) during a seven-day period. Fifty seeds were pooled in each replicate ($n = 3$). Values are mean \pm standard error (SE), and error bars represent SE

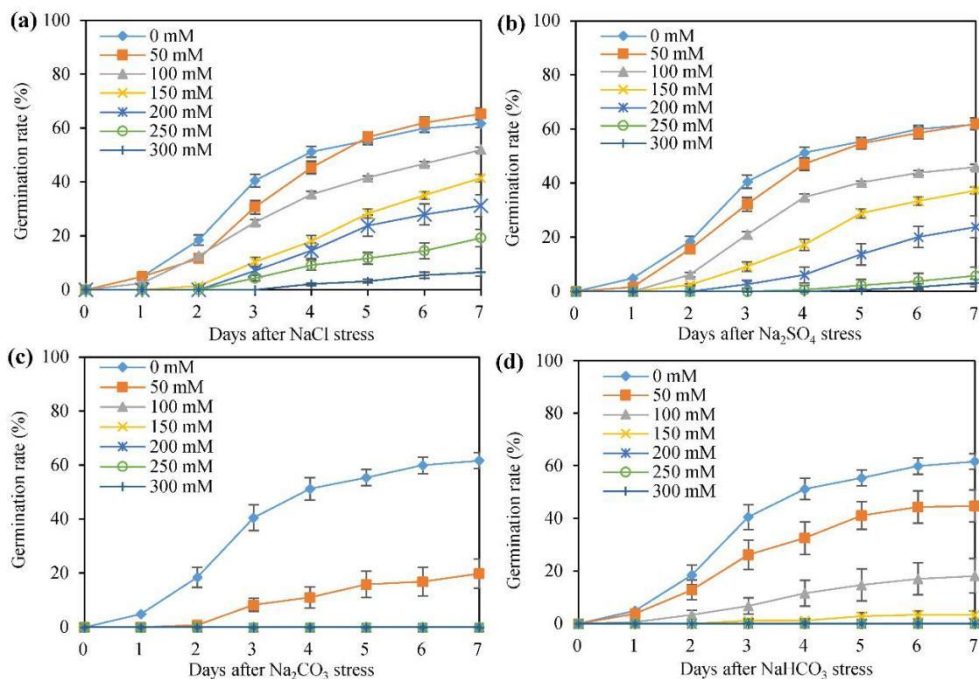


Figure 2. Time course of seed germination of the sainfoin cultivar “Italian” (IT) subjected to NaCl (a), Na₂SO₄ (b), Na₂CO₃ (c), and NaHCO₃ (d) in various concentrations (0, 50, 100, 150, 200, 250, and 300 mM) during a seven-day period. Fifty seeds were pooled in each replicate ($n = 3$). Values are mean \pm standard error (SE), and error bars represent SE

GS had a higher rate of seed germination than IT under both normal (no added salts) and saline treatments. At the end of the experiment on the seventh day, the highest germination rate under NaCl exposure for both cultivars occurred with the 50 mM NaCl treatment, followed by 100 mM in GS and 0 mM in IT. Germination rates were significantly reduced by higher salt concentrations in both GS (200, 250, and 300 mM) and IT (100, 150, 200, 250, and 300 mM) compared to control (0 mM) (Figs. 1a and 2a). Under Na₂SO₄ treatments, the concentration of 50 mM did not affect germination rate in either GS or IT, while higher concentrations (100–300 mM) significantly decreased germination rate in both cultivars compared to the control (0 mM) (Figs. 1b and 2b). Under 50 mM Na₂CO₃ treatment, GS began to germinate on the first day, while IT did not begin to germinate until the third day. By the seventh day, the 50 mM concentration of Na₂CO₃ significantly reduced germination rates of GS and IT by 52.9% and 67.9%, respectively, compared to the 0 mM control concentration, whereas high concentrations (100–300 mM) completely inhibited germination in both cultivars (Figs. 1c and 2c). Under NaHCO₃ treatment, germination rates were reduced significantly both in GS and IT by 50 mM concentration over a seven-day period compared to the 0 mM control. Exposure to 100 mM NaHCO₃ observably decreased germination rates in GS and IT to levels significantly lower than at a concentration of 50 mM. Both cultivars maintained lower germination rates when exposed to 150 mM NaHCO₃, and germination was almost completely inhibited under high concentrations (200, 250, and 300 mM) of NaHCO₃ throughout the seven days of treatment (Figs. 1d and 2d).

The effects of salt exposure on relative germination rate in the two cultivars were further analyzed. Under neutral salts (NaCl and Na₂SO₄), the relative germination rates both in GS and IT remained unchanged at 50 mM, but relative germination rate decreased significantly with increasing salt concentration from 100 to 300 mM (Fig. 3a, b).

Interestingly, the relative germination rate of GS was higher than IT under both NaCl and Na₂SO₄ treatments. Furthermore, Na₂SO₄ had a stronger inhibitory effect on seed germination than NaCl, especially at high concentrations. Under alkaline salts (Na₂CO₃ and NaHCO₃), the increasing of salt concentration reduced significantly the relative germination rates, reaching zero at 100 mM Na₂CO₃ and 200 mM NaHCO₃ (Fig. 3c, d), indicating that Na₂CO₃ more strongly inhibited seed germination than NaHCO₃. The relative germination rate of GS was significantly higher than that of IT when exposed to 50 and 100 mM NaHCO₃ (Fig. 3d). Moreover, the results of the two-way ANOVA showed that the effects of salinity type, salinity concentration, and their interaction on germination rates were significant in GS. However, in IT, both salt type and salt concentration had significant effects on germination of IT, but the interaction between salt type and salt concentration was not significant (Table 1).

Table 1. Two-way ANOVA of effects of salinity type and salinity concentration on germination rate and lengths of the hypocotyl and radicle in two sainfoin cultivars, “Gansu” (GS) and “Italian” (IT), exposed to salinity stress

Variable and source of variation	GS			IT		
	Germination rate	Hypocotyl length	Radicle length	Germination rate	Hypocotyl length	Radicle length
Salinity type (df = 3)	24.685**	14.698**	27.350**	7.886**	29.865**	1.036
Salinity concentration (df = 6)	62.195**	54.182**	123.842**	62.236**	63.289**	23.219**
Salinity type × concentration (df = 18)	3.130**	6.363**	3.310**	1.536	4.466**	1.345

P* < 0.05; *P* < 0.01

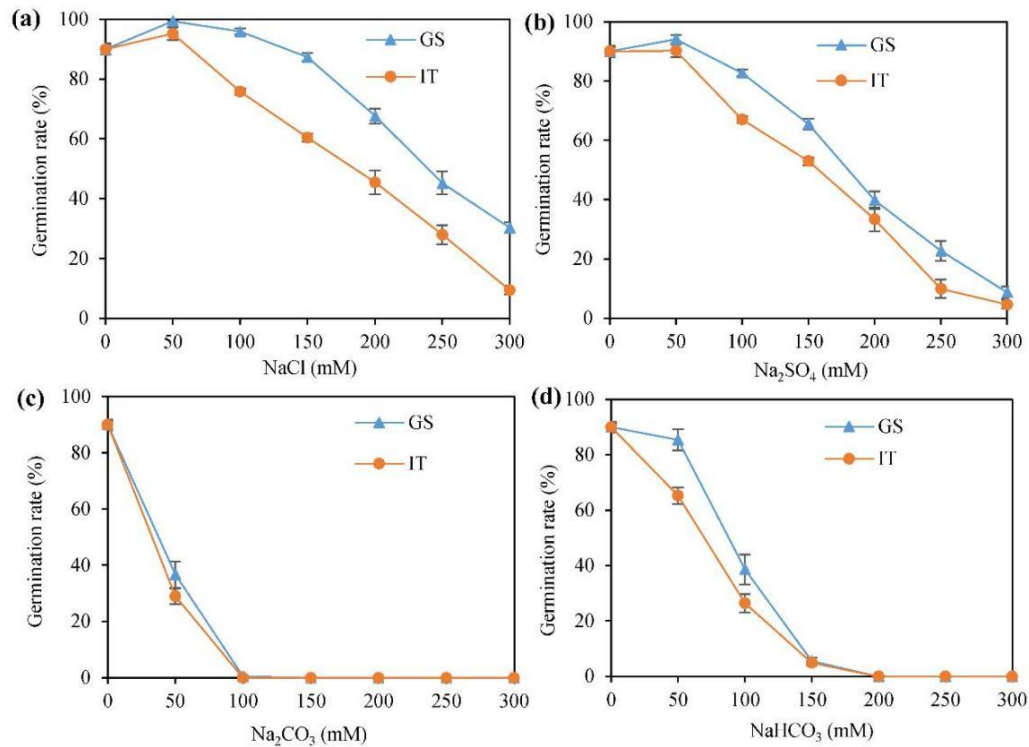


Figure 3. Germination rates of seeds in two sainfoin cultivars “Gansu” (GS) and “Italian” (IT) after subjected with NaCl (a), Na₂SO₄ (b), Na₂CO₃ (c), and NaHCO₃ (d) of various concentrations (0, 50, 100, 150, 200, 250, and 300 mM) for 7 d. Fifty seeds were pooled in each repeat (n = 3). Values are mean ± standard error (SE) and error bars represent SE

Effect of neutral and alkaline salts on growth of hypocotyl and radicle in two sainfoin cultivars during seed germination

To explore effects of different kinds of salts on seedling growth after seed germination, the lengths of hypocotyl and radicle were measured at the end of the 7-day salt exposure. As shown in *Table 2*, under neutral salts, all concentrations (50-300 mM) significantly reduced the lengths of hypocotyl and radicle in both GS and IT compared to the control (0 mM).

At the same salt concentration, the reduction of hypocotyl and radicle lengths by NaCl was less than by Na₂SO₄ in both cultivars. For example, at 300 mM NaCl and Na₂SO₄, hypocotyl length in GS was reduced by 89.9% and 94.9%, respectively, compared to control (0 mM). Under Na₂CO₃ stress, the minimum concentration needed to completely inhibit growth of both the hypocotyl and the radicle was 150 mM in GS, but only 100 mM in IT. However, under NaHCO₃ stress, the minimum concentration to completely inhibit growth was 200 mM in both cultivars (*Table 2*). The results of a two-way ANOVA showed that the effects of salt type, salt concentration, and the salt type × concentration interaction were significant for both hypocotyl length and radicle length in GS, but only for hypocotyl length in IT. Salinity type and salinity concentration had significant effects on radicle length in IT, but their interactive effect was not significant (*Table 1*). Further analysis showed that the hypocotyl and radicle growth under salinity stress varied among cultivars, salinity types, and salinity concentrations (*Table 3*).

Table 2. Effects of NaCl, Na₂SO₄, Na₂CO₃, and NaHCO₃ of various concentrations (0, 50, 100, 150, 250, and 300 mM) on growth of hypocotyl and radicle after seed germination in two sainfoin cultivars, “Gansu” (GS) and “Italian” (IT)

Stress		GS		IT	
Salinity type	Concentration (mM)	Hypocotyl length (mm)	Radicle length (mm)	Hypocotyl length (mm)	Radicle length (mm)
NaCl	0	26.33 ± 6.46 ^a	82.56 ± 10.67 ^a	24.00 ± 5.61 ^a	74.78 ± 15.72 ^a
	50	14.22 ± 7.64 ^b	57.78 ± 12.57 ^b	14.78 ± 3.59 ^b	59.00 ± 8.27 ^b
	100	5.00 ± 1.93 ^c	49.56 ± 6.24 ^c	7.33 ± 3.87 ^c	41.67 ± 8.07 ^c
	150	5.00 ± 3.16 ^c	41.00 ± 7.22 ^d	7.33 ± 4.24 ^c	37.22 ± 8.75 ^c
	200	3.33 ± 0.50 ^c	25.67 ± 3.70 ^e	3.78 ± 1.39 ^{cd}	19.44 ± 6.38 ^d
	250	3.33 ± 0.86 ^c	10.33 ± 5.07 ^f	3.11 ± 0.92 ^d	11.44 ± 6.12 ^{de}
	300	2.67 ± 1.00 ^c	6.11 ± 2.36 ^f	1.22 ± 0.44 ^d	1.78 ± 0.83 ^e
Na ₂ SO ₄	0	26.33 ± 6.46 ^a	82.56 ± 10.67 ^a	20.67 ± 2.87 ^a	72.00 ± 12.41 ^a
	50	12.22 ± 2.22 ^b	45.78 ± 10.45 ^b	8.00 ± 4.47 ^b	47.17 ± 7.80 ^b
	100	7.11 ± 3.01 ^c	39.89 ± 7.18 ^b	4.00 ± 1.41 ^c	35.17 ± 7.78 ^b
	150	3.44 ± 0.88 ^d	26.11 ± 3.75 ^c	2.83 ± 0.75 ^c	25.5 ± 7.71 ^{bc}
	200	3.11 ± 1.26 ^d	14.44 ± 7.41 ^d	2.83 ± 0.75 ^c	15.5 ± 1.97 ^{cd}
	250	2.11 ± 0.60 ^d	9.56 ± 3.84 ^{de}	2.75 ± 1.25 ^c	5.75 ± 1.89 ^{de}
	300	1.33 ± 0.50 ^d	4.00 ± 1.22 ^e	1.00 ± 0.00 ^c	2.00 ± 1.15 ^e
Na ₂ CO ₃	0	24.00 ± 1.87 ^a	74.89 ± 5.22 ^a	24.00 ± 1.87 ^a	74.89 ± 7.29 ^a
	50	10.88 ± 2.31 ^b	11.00 ± 1.82 ^b	10.44 ± 0.95 ^b	10.22 ± 3.59 ^b
	100	1.00 ± 0.00 ^c	1.33 ± 0.33 ^c	-	-
	150	-	-	-	-
NaHCO ₃	0	24.00 ± 1.87 ^a	74.89 ± 5.22 ^a	24.00 ± 1.87 ^a	74.89 ± 7.29 ^a
	50	18.75 ± 1.50 ^{ab}	18.13 ± 2.02 ^b	13.67 ± 1.84 ^b	11.67 ± 2.72 ^b
	100	15.71 ± 1.14 ^{bc}	5.71 ± 0.80 ^c	17.00 ± 1.22 ^b	4.33 ± 1.08 ^b
	150	10.00 ± 1.22 ^c	4.50 ± 0.95 ^c	4.40 ± 0.92 ^c	6.00 ± 1.54 ^b
	200	-	-	-	-

Columns with different lowercase letters indicate significant differences at $P < 0.05$ (Duncan's test)

Table 3. A three-way ANOVA analysis of effects of salinity types, concentrations and cultivars on germination rates of seeds, and lengths of hypocotyls and radicles in sainfoin exposed to salinity stresses

	Fv	Fs	Fc	Fv × s	Fv × c	Fs × c	Fv × s × c
df	1	3	6	3	6	18	18
Germination rate	318.878 ^{**}	56.006 ^{**}	198.717 ^{**}	6.746 ^{**}	2.123 [*]	6.956 ^{**}	1.791 [*]
Radicle length	0.221	33.282 ^{**}	111.598 ^{**}	7.848 ^{**}	2.125 [*]	8.340 ^{**}	2.187 [*]
Hypocotyl length	0.355	8.523 ^{**}	76.845 ^{**}	2.326	2.002	1.984 [*]	1.410

Fv, comparison of two sainfoin cultivars; Fs, comparison of two types of salinity; Fc, comparison of four concentrations of salinity; Fs × c, comparison of two kinds of salinity and four concentrations of salinity; Fv × c, comparisons of two cultivars and four concentrations of salinity; Fs × v × c, comparisons of two cultivars, two kinds of salinity, and four concentrations of salinity

* $P < 0.05$; ** $P < 0.01$

Seed germination recovery in two sainfoin cultivars after different types of salts stresses

To evaluate how harmful different types of salts and different salt concentrations were on seeds, the seeds ungerminated after seven days of salt treatment were transferred to distilled water, and germination recovery was tested. As shown in

Figure 4, after Na_2SO_4 exposure, germination recovery increased with increasing salt concentration from 50 to 300 mM in both cultivars, and the amount of increase was significantly less in IT than in GS.

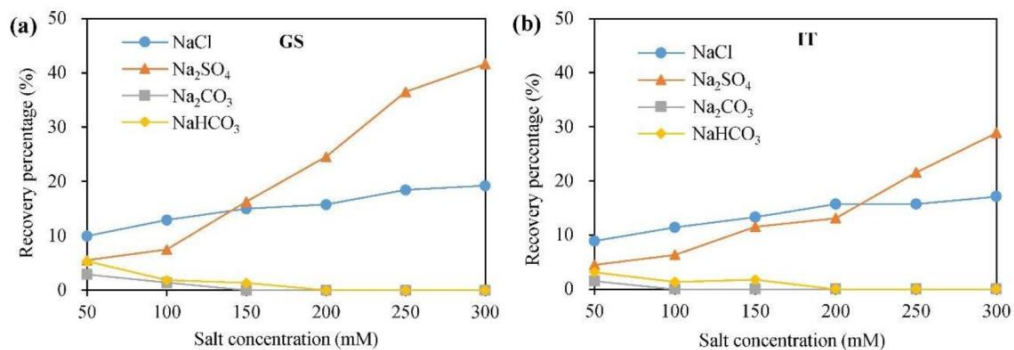


Figure 4. Recovery germination of seeds in two sainfoin cultivars “Gansu” (GS) (a) and “Italian” (IT) (b) subjected with NaCl, Na_2SO_4 , Na_2CO_3 , and NaHCO_3 of various concentrations (50, 100, 150, 200, 250, and 300 mM) for 7 d

After NaCl stress, the recovery germination percentage (RGP) maintained the stable levels with increasing salt concentration in both GS and IT. Compared to the two neutral salts, after exposure to two alkaline salts, RGP significantly declined with increasing salt concentration. Although germination recovery also occurred in alkaline salt treatments, RGP was very low, and the maximum RGP was only 5%. The RGP reached zero when the concentration of Na_2CO_3 was 100 and 150 mM in IT and GS, respectively. However, recovery germination did not occur in either GS or IT when concentrations of NaHCO_3 exceeded 200 mM (Fig. 4a, b). These results suggest that alkaline salinities are more harmful to seeds of both sainfoin cultivars than neutral salinities.

Threshold and critical salt concentrations for the germination of seeds and growth of hypocotyl and radicle in two sainfoin cultivars under different types of salts stresses

As shown in Table 4, both threshold and critical salinity concentrations for seed germination were relatively higher under neutral salts than under alkaline salts for both cultivars. Under neutral salts, GS exhibited higher threshold concentrations of 200 mM NaCl and 150 mM Na_2SO_4 and higher critical concentrations of 250 mM NaCl and 200 mM Na_2SO_4 , whereas IT showed relatively lower threshold and critical concentrations. Under alkaline salts, GS displayed the same threshold and critical concentrations as IT in both Na_2CO_3 and NaHCO_3 conditions (Table 4).

Discussion

The different effects of neutral and alkaline salinities on seed germination and recovery of two sainfoin cultivars

Both neutral and alkaline salts inhibited seed germination, with germination significantly reduced with increasing salt concentrations (Figs. 1 and 2). For example, the germination rate of GS under 50 mM of neutral salts was as high as the control

(0 mM) (>80%) (Fig. 1a, b), and the length of the radicle and hypocotyl exceeded 12 and 45 mm, respectively (Table 2). However, few seeds germinated (<30%) at a concentration of 300 mM (Fig. 1a, b), and the lengths of the hypocotyl and radical at this high salt concentration were reduced by more than 90% (Table 2). In alkaline salinities, hypocotyl and radicle length were reduced to zero (Table 2). Similar results were also found in *Haloxylon ammodendron* (Huang et al., 2003), in which the germination rate gradually declined with increasing salinity concentrations.

Table 4. The threshold and critical salt concentration for the seed germination, and the hypocotyl and radicle growth in two sainfoin cultivars “Gansu” (GS) and “Italian” (IT) exposed to different salts stresses

Cultivars	Salinity	Threshold salinity concentration (mM)			Critical salinity concentration (mM)		
		Germination	Hypocotyl	Radicle	Germination	Hypocotyl	Radicle
GS	NaCl	200	50	50	250	100	150
	Na ₂ SO ₄	150	50	50	200	50	100
	Na ₂ CO ₃	50	50*	50*	50	50	50
	NaHCO ₃	100	100	50*	100	100	50
IT	NaCl	150	50	50	250	100	150
	Na ₂ SO ₄	100	50	100	200	50	100
	Na ₂ CO ₃	50	50	50*	50	50	50*
	NaHCO ₃	100	50	50*	100	150	50

Threshold salinity concentration is defined as the highest level of salinity resulting in non-significant decrease, and the critical salinity concentration is defined as the level of salinity resulting in a 50% decrease of seed germination or lengths of hypocotyls and radicles. “*” represents significant decrease compared to the control from 50 mM salinity concentration

It is well-known that neutral salts and alkaline salts impose distinct types of stresses (Shi and Sheng, 2005). Plants exposed to alkaline conditions experienced not only ionic stress but also high pH stress, which aggravates the effects of ionic toxicity and osmotic stress on seeds and damages the seed embryo (Yang et al., 2009). In the present study, the differences between neutral and alkaline salinities were further investigated. It is clear that alkaline salinity was more harmful than neutral salinity to sainfoin seed germination and seedling growth (Table 2). Both GS and IT cultivars had higher germination under neutral salinities (NaCl and Na₂SO₄) than under alkaline salinities (NaHCO₃ and Na₂CO₃). Under Na₂CO₃, seeds germinated only at a concentration of 50 mM. Similarly, under NaHCO₃ stress, germination did not occur at concentrations exceeding 150 mM. However, at even 300 mM concentrations of NaCl and Na₂SO₄ seeds still germinated (Figs. 1 and 2). Additionally, sainfoin exhibited a relatively higher threshold concentration of neutral salts than alkaline salts during germination. The threshold concentrations of NaCl and Na₂SO₄ for GS germination were 200 and 150 mM, while the corresponding thresholds of alkaline salts (Na₂CO₃ and NaHCO₃) were 50 and 100 mM, respectively. However, for germination of the IT cultivar, the critical concentrations of NaCl and Na₂SO₄ were 250 and 200 mM, while the corresponding critical concentrations of alkaline salts (Na₂CO₃ and NaHCO₃) were 50 and 100 mM, respectively (Table 4). These results suggest that damage caused by alkaline salts is more serious than those caused by neutral salts. In previous studies, it was observed that the damage due to alkaline salts are often more complex, and the

process and degree of damage, as well as the breakdown of ecological performance, are more severe than those due to neutral salts (Yang et al., 2009).

In the neutral salt treatments, the recovery germination percentage increased with increasing salt concentration (Fig. 4). For instance, the recovery percentages in GS increased from 5% to 40% under Na₂SO₄, with the maximum recovery germination percentage occurring after exposure to 300 mM, indicating that neutral salts only temporarily inhibited seed germination in sainfoin. However, under alkaline salt stress, the recovery germination percentage sharply decreased with increasing salt concentrations. In addition, both cultivars exhibited lower recovery germination percentage in alkaline conditions compared to neutral salinity conditions (Fig. 4). These results implied that alkaline salinity not only temporarily inhibited seed germination but also led to prolonged toxicity.

The different tolerance to salt stresses in two sainfoin cultivars

Both neutral and alkaline salinity stresses led to reduced germination rate with increasing salt concentration. The reduction in germination was proportional to the concentration of Na⁺, with more Na⁺ leading to lower rates of germination (Hu et al., 2018). Under neutral salt conditions, the germination curve of 250 mM in NaCl almost coincided with that of Na₂SO₄ at 200 mM in both GS and IT. Similarly, under alkaline conditions, the germination curve of 50 mM in Na₂CO₃ was almost the same as that of NaHCO₃ at 100 mM (Figs. 1a, b and 2a, b). These results demonstrated that Na⁺ might be toxic to seed germination. Na⁺ has been reported to have a physiological role in seedlings of sugar beet (Wu et al., 2015b), and high absorption of Na⁺ inhibited and decreased germination rate (Carpóczy et al., 2009). Toxicity of Na⁺ may therefore be the main cause of reduced germination with increasing salinity concentration.

Salt tolerance has been shown to differ during seed germination and vary among different plant varieties (Hu et al., 2018). Although GS and IT exhibited a very similar appearance and displayed the same patterns of response to saline and alkaline stresses, there were nonetheless obvious differences in salt tolerance between the two cultivars. GS was more tolerant to neutral salt stress during germination, as the germination rate showed a relatively higher threshold concentration compared to IT (Table 4). Additionally, the radicles and hypocotyls of seedlings grown under NaCl and Na₂SO₄ were also longer in GS than in IT. These results confirmed that GS was more tolerant to NaCl and Na₂SO₄. However, both GS and IT experienced sharp reductions in germination at higher concentrations of alkaline salts (Fig. 3), which was exemplified by the lower critical concentration at 50 mM Na₂CO₃ and at 100 mM NaHCO₃ (Table 4). These results implied that sainfoin is extremely sensitive to alkaline conditions.

It is well documented that the role of the seed coat is to protect the embryo from harmful environmental conditions (Wierzbicka and Obidzińska, 1998; Yang et al., 2012). The effect of abiotic stress on seed germination of different crop cultivars depends upon different seed structures (Wierzbicka and Obidzińska, 1998; Ashraf and Foolad, 2005). In general, a solid seed coat can protect the seed embryo from salt toxicity, particularly because of its wide variability in structure compared to other tissues or organs (Wierzbicka and Obidzińska, 1998). The seed coat, therefore, is the major barrier to salinity stress, and it prevents embryo toxicity prior to seed coat degradation by the germinating embryonic roots (Hu et al., 2018). As the two sainfoin cultivars have similar structures of seeds, the reason for the stronger salinity tolerance

of GS compared to that of IT may be attributed to its higher quality seeds (i.e. the heavier mass of 100 seeds), which can hold more dry matter and more energy in the seed embryo.

Conclusions

Different types of salts inhibited seed germination and growth of hypocotyls and radicles, with increasing inhibition with increasing salt concentrations. Salts affected sainfoin germination and early growth in the following order: $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4 > \text{NaCl}$. Neutral salinity only temporarily inhibited germination, whereas alkaline salinity temporarily inhibited germination during exposure but also proved toxic to sainfoin seeds even after removal of the salts. Thus, the effects of neutral salinity and alkaline salinity were different, and damage due to alkaline salinity was more severe than that of neutral salinity. GS seeds germinated and grew more rapidly compared to IT seeds under saline-alkaline stress, suggesting that GS is more salt-tolerant. However, the molecular mechanisms underling the response of sainfoin to saline-alkaline stress need to be addressed in the future research.

Acknowledgements. This research was funded by the National Natural Science Foundation of China, grant number 31860404; Science and Technology Department of Gansu Province, grant number 21YF5FA082; Lanzhou Science and Technology Bureau, grant number 2017-4-94. We would like to thank all the people involved in this project and the reviewers who provided constructive comments. We would also like to thank Dr. Adam Roddy at Yale University for his assistance with English language and grammatical editing.

REFERENCES

- [1] Abbasdokht, H.; Gholami, A.; Asghari, H. (2014): Halopriming and hydropriming treatments to overcome salt and drought stress at germination stage of corn (*Zea mays* L.). – *Desert* 19: 27-34.
- [2] Ahmadvand, G.; Soleimani, F.; Saadatian, B.; Pouya, M. (2012): Effects of seed priming on germination and emergence traits of two soybean cultivars under salinity stress. – *International Journal of Applied and Basic Sciences* 3: 234-241.
- [3] Albuquerque, F. E.; Carvalho, N. M. (2003): Effect of the type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soybean (*Glycine max* (L.) Merrill) and maize (*Zea mays* L.) seeds with different levels of vigor. – *Seed Science & Technology* 31: 465-479.
- [4] Ashraf, M.; Foolad, M. R. (2005): Pre-sowing seed treatment - A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. – *Advances in Agronomy* 88: 223-271.
- [5] Baldinger, L.; Hagmüller, W.; Minihuber, U.; Matzner, M.; Zollitsch, W. (2014): Sainfoin seeds in organic diets for weaned piglets-utilizing the protein-rich grains of a long-known forage legume. – *Renewable Agriculture and Food Systems* 31: 12-21.
- [6] Carpýcý, E. B.; Celýk, N.; Bayram, G. (2009): Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. – *African Journal of Biotechnology* 8: 4918-4922.
- [7] Cirujeda, A.; Marí, A. I.; Murillo, S.; Aibar, J.; Pardo, G.; Solé-Senan, X. O. (2019): May the inclusion of a legume crop change weed composition in cereal fields? Example of sainfoin in Aragon (Spain). – *Agronomy* 9: 134.

- [8] Gebremedhn, Y.; Berhanu, A. (2013): The role of seed priming in improving seed germination and seedling growth of maize (*Zea mays* L.) under salt stress at laboratory conditions. – African Journal of Biotechnology 12: 6484-6490.
- [9] Gorai, M.; Neffati, M. (2007): Germination responses of *Reaumuria vermiculata* to salinity and temperature. – Annals of Applied Biology 151: 53-59.
- [10] Hu, H.; Liu, H.; Liu, F. (2018): Seed germination of hemp (*Cannabis sativa* L.) cultivars responds differently to the stress of salt type and concentration. – Industrial Crops and Products 123: 254-261.
- [11] Huang, Z.; Zhang, X.; Zheng, G.; Gutterman, Y. (2003): Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*. – Journal of Arid Environments 55: 453-464.
- [12] Idris, Y. A.; Ali, S. A. M. (2015): Response of maize (*Zea mays* L.) to sodium chloride stress at early growth stages. – International Journal of Agronomy and Agricultural Research 6: 68-74.
- [13] Khalil, C.; Houssein, B. E.; Hassan, B.; Fouad, M. (2016): Comparative salt tolerance study of some acacia species at seed germination stage. – Asian Journal of Plant Sciences 15: 66-74.
- [14] Li, X.; Zhao, W. Z. (2018): Effects of salt-alkaline mixed stresses on seed germination and seedling growth of *Bassia dasyphylla* in desert region. – Journal of Desert Research 38: 300-306.
- [15] Lu, S. W.; Li, T. L.; Jiang, J. (2010): Effects of tomato fruit under Na⁺-salt and Cl⁻-salt stresses on sucrose metabolism. – African Journal of Agricultural Research 5: 2227-2231.
- [16] Mohajer, S.; Taha, R. M.; Lay, M. M.; Esmaceli, A. K.; Khalili, M. (2014): Stimulatory effects of gamma irradiation on phytochemical properties, mitotic behaviour, and nutritional composition of sainfoin (*Onobrychis viciifolia* Scop.). – The Scientific World Journal 2014: 1-9.
- [17] Munns, R.; Tester, M. (2008): Mechanisms of salinity tolerance. – Annual Review of Plant Biology 59: 651-81.
- [18] Muscolo, A.; Sidari, M.; Anastasi, U.; Santonoceto, U.; Maggio, A. (2014): Effect of PEG-induced drought stress on seed germination of four lentil genotypes. – Journal of Plant Interactions 9(1): 354-363.
- [19] Niu, Y.; Yang, X. Y.; Dai, C. F.; Wang, B. W.; Ren, G. L.; Wu, J. L.; Wang, F. B.; Chen, X. H. (2018): Related indices selection of soybean salt tolerance at germination and seedling stages. – Soybean Science 37: 216-223.
- [20] Okcu, M.; Topaloglu, F. N. (2019): Effect of different gibberellic acid doses treatment on seed germination of wild sainfoin (*Onobrychis vichfolia*). – Fresenius Environmental Bulletin 28: 1062-1068.
- [21] Partheeban, C.; Chandrasekhar, C. N.; Jeyakumar, P.; Ravikesavan, R.; Gnanam, R. (2017): Effect of PEG induced drought stress on seed germination and seedling characters of maize (*Zea mays* L.) genotypes. – International Journal of Current Microbiology and Applied Sciences 6: 1095-1104.
- [22] Petrovic, G.; Jovicic, D.; Nikolic, Z.; Tamindzic, G.; Ignjatov, M.; Milosevic, D.; Milosevic, B. (2016): Comparative study of drought and salt stress effects on germination and seedling growth of pea. – Genetika 48: 373-381.
- [23] Radic, V.; Balalic, I.; Jacimovic, G.; Nastasic, A.; Savic, J.; Marjanovic-Jeromela, A. (2019): Impact of drought and salt stress on seed germination and seedling growth of maize hybrids. – Genetika 51: 743-756.
- [24] Shi, D.; Sheng, Y. (2005): Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. – Environmental and Experimental Botany 54: 8-21.
- [25] Wierzbicka, M.; Obidzińska, J. (1998): The effect of lead on seed imbibition and germination in different plant species. – Plant Science 137: 155-171.

- [26] Wu, G. Q.; Shui, Q. Z.; Wang, C. M.; Zhang, J. L.; Yuan, H. J.; Li, S. J.; Liu, Z. J. (2015a): Characteristics of Na⁺ uptake in sugar beet (*Beta vulgaris* L.) seedlings under mild salt conditions. – *Acta Physiologiae Plantarum* 37: 1-13.
- [27] Wu, H.; Shabala, L.; Liu, X.; Azzarello, E.; Zhou, M.; Pandolfi, C.; Pandolfi, C.; Chen, Z.; Bose, J.; Mancuso, S.; Shabala, S. (2015b): Linking salinity stress tolerance with tissue-specific Na⁺ sequestration in wheat roots. – *Frontiers in Plant Science* 6: 71.
- [28] Wu, G. Q.; Jia, Z.; Liu, H. L.; Wang, C. M.; Li, S. J. (2017): Effect of salt stress on growth, ion accumulation, and distribution in sainfoins (*Onobrychis viciaefolia*) seedlings. – *Pratacultural Science* 34: 1661-1668.
- [29] Wu, G. Q.; Li, H., Zhu, Y. H., Li, S. J. (2021): Comparative physiological response of sainfoin (*Onobrychis viciaefolia*) seedlings to alkaline and saline-alkaline stress. – *Journal of Animal and Plant Sciences*. 31(4): 1028-1035.
- [30] Yang, C. W.; Zhang, M. L.; Liu, J.; Shi, D. C.; Wang, D. L. (2009): Effects of buffer capacity on growth, photosynthesis, and solute accumulation of a glycophyte (wheat) and a halophyte (*Chloris virgata*). – *Photosynthetica* 47: 55-60.
- [31] Yang, X.; Baskin, J. M.; Baskin, C. C.; Huang, Z. (2012): More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. – *Perspectives in Plant Ecology Evolution & Systematics* 14: 434-442.
- [32] Zahra, K.; Ifar, M.; Motamedi, M. (2011): Effects of NaCl salinity on maize (*Zea mays* L.) at germination and early seedling stage. – *African Journal of Biotechnology* 11: 298-304.
- [33] Zhang, H.; Zhang, G.; Lu, X.; Zhou, D.; Han, X. (2014): Salt tolerance during seed germination and early seedling stages of 12 halophytes. – *Plant and Soil* 388: 229-241.